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# Interactions of VAM fungi, pesticides and crops

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## Interactions of VAM fungi, pesticides, and crops

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### Budget

\$20,000 for year one  
\$20,000 for year two

### Background

Vesicular arbuscular mycorrhizal fungi (VAM) are beneficial root symbionts of most crops, including corn and soybeans. These fungi colonize plant roots, facilitating nutrient exchange between the plant and the fungus. The plant supplies carbon for fungal growth; in turn, the VAM enhances uptake of relatively immobile nutrients such as phosphorus (P). The network of external fungal filaments, or hyphae (see photos below), extends outside the root up to several centimeters in the soil, allowing the fungus access to soil P otherwise unavailable to the plant.

Most research on VAM fungi has focused on how they affect crop performance. However, because carry-over of herbicide residue interferes with crop rotations and replant situations, this project was undertaken to study the possible interactions among VAM fungi, crops, and herbicides. Because VAM fungi may increase plant uptake of herbicide much as they do P uptake, crop injury could result. Moreover, it is possible that VAM fungi play a role in degrading herbicides or protecting

some plants from herbicide injury by production of certain compounds.

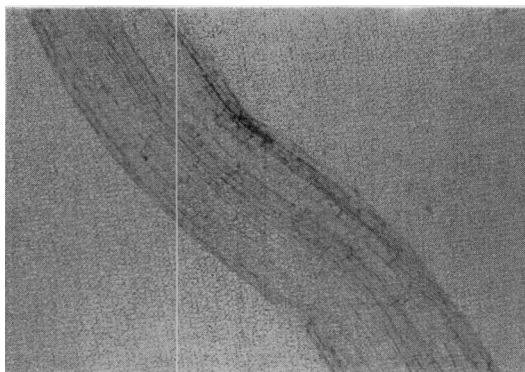
Thus, the overall goal of this project was to determine whether VAM fungi contribute to the root uptake of herbicides. VAM fungi's role in degradation of herbicides in the root zone of the soil was also studied.

### Specific objectives included

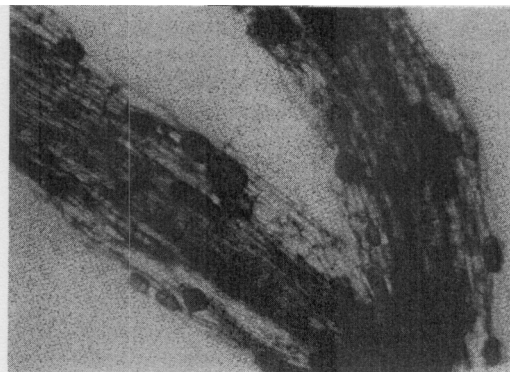
- (1) determining the effects of VAM fungi in the growth of soybeans and corn in soils treated with atrazine and trifluralin at rates designed to stimulate herbicide carry-over in soils that had low and high available P,
- (2) quantifying the direct uptake of herbicide by VAM fungi,
- (3) assessing the effect of these herbicides on VAM fungal infection, and
- (4) assessing the rhizosphere degradation of herbicide by VAM fungi.

### Approach and methods

The herbicides atrazine (widely used on corn) and trifluralin (widely used on soybeans) were selected for use in these experiments because



*This photo shows a root with no VAM fungi colonization. The vascular bundles can be seen in the slightly darker core; the fungus colonizes in the surrounding cortex, producing the hyphae (see photo at right).*



*This photo shows the VAM fungal structures inside the root cortex. The spherical shapes are the storage vesicles of the VAM fungus; the threadlike filaments are the hyphae.*

of the major role they play in Iowa agriculture. For that reason as well, experiments were conducted in soils treated with these two herbicides at rates that simulated herbicide carry-over. These interactions were studied in soils both low and high in plant available P. Two methods of evaluating herbicide uptake allowed comparisons of the efficiency of the two different root systems under conditions that eliminated the influence of other environmental factors. The exact role of VAM fungal hyphae in herbicide uptake was also studied by using a system designed to allow only the hyphae access to the herbicide-treated soil.

**VAM effects on crop response to herbicides:** Randomized greenhouse experiments were established to determine plant growth and VAM colonization of roots in soils treated with atrazine or trifluralin. Soil containing a well-established, native VAM population of several species was collected from the borders of long-term corn plots that had been maintained at a low P status. Corn and soybean plants were planted, fertilized, and observed periodically through harvest at seven weeks. Shoots and root systems were harvested separately, and subsamples of the fine tertiary roots were stained to show VAM colonization, which was determined on a relative percentage basis. Three types of soil and soil-sand mixtures were prepared—one containing a native population of VAM fungi and soil microorganisms, one that was inoculated with the VAM fungus *Glomus epigaeum* and microorganisms, and one that contained no VAM fungi. These soils were then treated with the atrazine or trifluralin at rates that simulate herbicide carry-over. Soybeans were planted in the atrazine-treated soil and corn in the trifluralin-treated soil. Each treatment involved four replications.

In addition, the effect of trifluralin and atrazine on VAM formation was assessed in plants tolerant to these herbicides. Fungal colonization of soybeans and corn was measured in soil treated with atrazine and trifluralin, respectively. The effect of VAM on soybean growth in the presence of atrazine in high P soil was also investigated.

**Herbicide uptake by excised roots:** To compare the short-term rates of herbicide uptake by VAM-colonized and VAM-free root segments in solutions of herbicides, atrazine uptake studies were conducted using both corn and soybean roots; trifluralin uptake was measured only with soybean roots. Plants were harvested, shoot and leaf portions removed, and roots washed with care to remove soil yet avoid breaking the external hyphal network. The fine, actively growing root samples were exposed to herbicide for six different lengths of time in three replications to determine their degree of adsorption. Herbicide uptake was quantified by measuring radioactivity in roots.

**Herbicide uptake by whole root systems:** Short-term rates of herbicide uptake by intact mycorrhizal and non-mycorrhizal root systems in herbicide solutions were also compared, and atrazine uptake by soybean roots and trifluralin uptake by corn roots were measured. An additional treatment used sterile soil containing additional available P to compensate for the lack of mycorrhizae. The undisturbed root systems were placed in a herbicide solution and then analyzed to determine amount of adsorption.

**Herbicide uptake by external hyphae:** Herbicide uptake was also compared in mycorrhizal and non-mycorrhizal plants grown in special two-compartment containers designed to allow hyphal penetration (without roots) into a compartment containing herbicide-treated soil. This method was employed because movement of the herbicide into the plant would constitute unequivocal evidence of hyphal uptake and translocation from soil. A screen separating the two compartments prevented root entry but allowed hyphal penetration to the compartment containing the herbicide. Soil was kept watered approximately at field capacity; however, care was taken that water did not flow between compartments. Plants were harvested after eight weeks, a growth period sufficient for development of external VAM hyphae. Shoot and leaf portions were harvested first; again, care was taken not to damage hyphal development. Soil

was sampled for herbicide concentration and fungal development.

**Rhizosphere degradation of atrazine:** Atrazine degradation rates were compared in the rhizospheres of mycorrhizal and non-mycorrhizal corn plants. Three replicate plants from each treatment were harvested at five intervals after planting. Soil was freed from intact root systems and analyzed for atrazine and its metabolites.

## Findings

**VAM effect on crop response to herbicides:** Corn was tolerant of atrazine but susceptible to trifluralin. Atrazine is a photosynthetic inhibitor, but corn is able to hydrolyze it rapidly. Soybean is tolerant of trifluralin, but susceptible to atrazine. Trifluralin can cause stunting of lateral roots even in tolerant plants; in susceptible plants, it blocks both root and shoot elongation.

The VAM fungi enhanced growth of corn and soybean plants in soil that was low in available P. In the absence of VAM fungi, corn exhibited some stress due to atrazine treatment. VAM colonization of corn was reduced slightly by atrazine, but trifluralin had no effect on VAM infection.

Atrazine (applied to soybeans) and trifluralin (applied to corn) reduced growth of both plants at low doses. Atrazine at 0.5 and 1.0 ppm killed all soybean plants in the first experiment. These herbicide rates are more typical of those that would be found immediately following applications in farm fields. While these rates were not included in subsequent experiments, atrazine at 0.25 ppm also killed all soybean plants.

Soybean growth was significantly enhanced by the VAM fungi in untreated soil and at all levels of atrazine application. Growth of mycorrhizal soybean plants was 114% greater for plants inoculated with *G. epigaeum* at 0.1 mg/kg of atrazine. Growth reduction by atrazine in VAM-infected soybeans was nearly equivalent to that in non-mycorrhizal plants. In the first experiment, different results were obtained in that VAM-infected soybeans ap-

peared to be somewhat more affected by atrazine than non-mycorrhizal soybeans. Soybean responses to atrazine stress were comparable to those of plants grown in soil containing native populations of VAM fungi. Atrazine at 0.1 mg/kg soil reduced *G. epigaeum* colonization slightly, but atrazine had no effect on P uptake. *The concentration of P in mycorrhizal soybeans was two times higher than that of non-mycorrhizal plants—a clear indication that the positive effects of VAM fungi in promoting plant growth by increasing P uptake are more important than any negative effects due to herbicide uptake.*

Trifluralin caused substantial reductions in growth of corn. Corn plants colonized with native populations of VAM fungi exhibited greater tolerance to trifluralin than the *G. ^p/ga^wm*-inoculated non-mycorrhizal corn. Corn shoot and leaf P content was increased by both the indigenous mycorrhizal fungi and *G. epigaeum*, but trifluralin had less of an effect on shoot and leaf weights than did P content. *G. epigaeum* was more effective in increasing corn growth than the indigenous species of VAM fungi, both in terms of plant growth and in root colonization. Trifluralin had no effect on VAM formation.

An additional experiment to measure the soybean response to atrazine was conducted with higher available concentrations of soil P. When P was scarce, VAM fungi increased P uptake, which could have obscured the simultaneous uptake of herbicides. Moderate to high concentrations of available P are representative of the general P status of Iowa soils. Soybean growth was slightly less in VAM-infected plants at the higher atrazine concentrations. These trends are similar to those reported in other, related research. In these experiments, VAM root colonization was less than 15%, compared to colonization exceeding 60% in soil low in available P. The lower VAM formation probably reduced the network of external hyphae to a similar extent.

**Herbicide uptake by excised roots:** Uptake of atrazine and trifluralin by both mycorrhizal and non-mycorrhizal roots depended greatly on the herbicide concentration. Roots infected

with VAM consistently took up more atrazine than non-mycorrhizal roots. Atrazine absorption at one rate was 9 and 12 times higher than uptake at one-tenth the rate for VAM and non-VAM roots, respectively, after the longest time period, 24 hours.

Root tissue absorbed atrazine and trifluralin rapidly, accounting for 10 to 78% of the uptake during the first 30 minutes. Absorption at 24 hours was much higher than after six hours, indicating that the roots were not saturated.

Absorption of atrazine was significantly different at various stages of plant growth in VAM-infected roots (see Figs. 1 and 2). However, uptake was very similar in non-VAM roots at different stages of growth. In all the experiments, the actively growing tertiary root segments were collected for the adsorption assays, based on the assumption that these roots form the largest and most active part of the root system. This may have reduced the effects of root age in this study. Root diameter was measured; the resulting calculations of root volume were greater for the older VAM-infected roots, but the internal VAM infection was not significantly different in the different

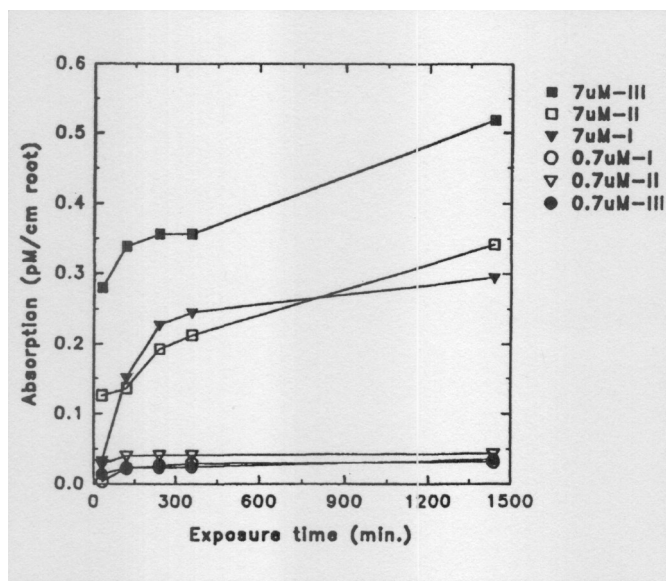
aged roots. The external hyphal length was not quantified, but an increased amount of extramatrical hyphae (those external to the root) was very evident. The higher uptake of atrazine by older VAM-infected roots is possibly due to the greater extramatrical hyphal network and thereby an increase in the active surface area for uptake. The VAM-infected roots showed greater uptake of atrazine than non-mycorrhizal roots, even after accounting for the differences in root volume between VAM and non-VAM treatments.

#### Herbicide uptake by whole root systems:

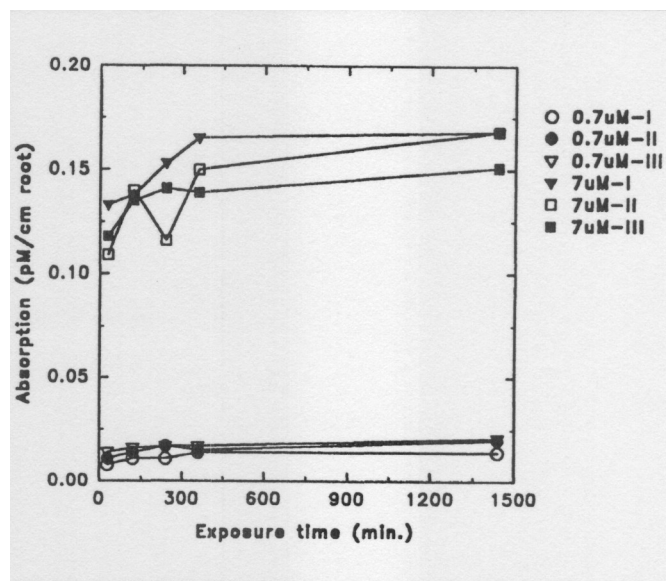
Absorption of atrazine and trifluralin by intact root systems of soybean and corn, respectively, were slightly increased by *G. epigaeum* infection. The uptake by VAM-infected root systems was greater than non-VAM systems, but there was high variability within the treatments. Measurements included large roots, which do not support active mycorrhizal infections.

#### Herbicide uptake by external hyphae:

Soybean and corn dry matter were significantly greater for plants infected with *G. epigaeum*. VAM-infected corn root systems were 1,456%



**Fig. 1.** Absorption of atrazine by *G. epigaeum*-infected corn roots at different stages of growth. I, II, and III represent absorption by roots after 6, 7, and 8 weeks of growth, respectively.



**Fig. 2.** Atrazine absorption by non-VAM corn roots at different stages of growth. I, II, and III represent absorption by roots after 6, 7, and 8 weeks of growth, respectively.

larger and soybeans 223% larger than non-VAM plants. *G. epigaeum* penetrated the screen vigorously into the soil containing the herbicides. Soybean roots were not as extensive as corn roots, but VAM infection was above 80% for both plants.

The *G. epigaeum* hyphal uptake of atrazine was evident in inoculated corn plants, but despite precautions, some contamination of the top soil with herbicide occurred in some of the non-VAM treatments, perhaps via volatilization or water movement across the screen.

The data show that VAM hyphal systems can remove and transfer atrazine residues to plants. There was no evidence of trifluralin uptake by the soybean mycorrhizae. Total herbicide uptake and accumulation in the plant was probably related to the extent of root development and hyphal penetration through the screen to the herbicide-treated soil. Root masses of corn plants in contact with the screen were significantly larger than for soybean plants; growth and root development of the latter were limited possibly by the low P status of the soil, rendering interpretation of the trifluralin uptake data difficult. The data on herbicide exchange and translocation was quantified in three separate layers for comparison purposes.

**Rhizosphere degradation of atrazine:** Figure 3 shows degradation of atrazine in the

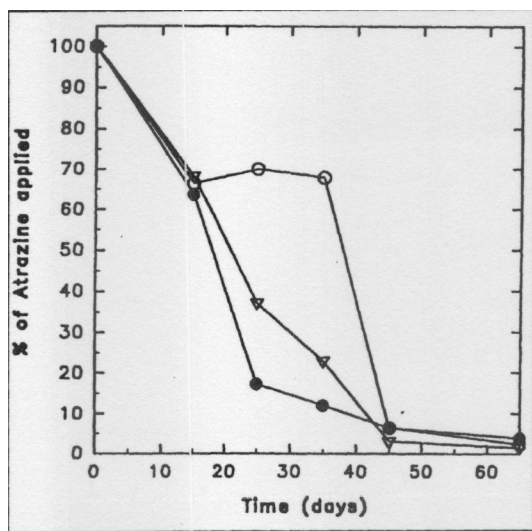
rhizosphere of corn. The initial atrazine degradation rate was slightly lower in the VAM-influenced rhizosphere, but the long-term effect was similar for all treatments, indicating that *G. epigaeum* is not directly involved in atrazine degradation and does not have a significant indirect effect on atrazine-degrading microorganisms in the rhizosphere. The external hyphal extension of *G. epigaeum* in the rhizosphere was difficult to quantify.

## Implications

This project demonstrated that atrazine reduced the VAM colonization of roots, indicating that atrazine was moderately toxic to *G. epigaeum*—but not sufficiently so to affect crop growth.

The project also showed that VAM fungi increase uptake of organic herbicides by plants in two ways: mycorrhizal plants are larger than non-mycorrhizal plants; thus they contact more herbicide. The VAM fungi also take up herbicide from the soil and translocate herbicide into the root. Thus, mycorrhizal plants take up more herbicide even when root volume differences are taken into account.

However, this increased uptake is of importance only when sensitive plants are exposed to herbicide residues. In agriculture, such situations arise due to the carry-over of herbicides to the following crops or after crop



**Fig. 3. Degradation of atrazine in the rhizosphere of corn infected with *G. epigaeum* (hollow circles), non-mycorrhizal plants (triangles), and non-mycorrhizal plants with additional phosphate (filled circles).**

failure, where farmers replant a different crop into soil treated with herbicide. Another situation in which plants are likely to encounter potentially phytotoxic herbicide residues is in the use of plants for bioremediating pesticide wastes.

These experiments simulated exposure to herbicides due to carry-over and found that mycorrhizal soybeans were similar to non-mycorrhizal plants in the response to herbicides. *Results indicated that the positive effects of VAM fungi in promoting plant growth (due to increased P uptake) were more important than*

*the negative effects due to increased herbicide uptake, and that VAM are only a minor agent affecting crop tolerance to herbicide residues.*

Although this work did not show statistically significant effects on plant growth in a situation simulating herbicide carry-over, plant damage from herbicides is controlled by VAM-herbicide interaction factors that were not fully assessed in this work.

This work has added to the knowledge base; research continues with support from the USDA-ARS, National Soil Tilth Laboratory.

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